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Detection of Metabolites by Frequency-Pulsed Electron Capture Gas-Liquid Chromatography in Serum and Cerebrospinal Fluid of a Patient with *Nocardia* Infection

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Serum (SR) and cerebrospinal fluid (CSF) from a patient suspected of having tuberculous meningitis were submitted to our laboratory for analysis by frequency-pulsed electron capture gas-liquid chromatography (FPEC GLC). The samples were tested for the presence of carboxylic acids, alcohols, hydroxy acids, and amines by methods described previously (C. C. Alley, J. B. Brooks, and D. S. Kellogg, Jr., J. Clin. Microbiol. 9:97–102, 1977; J. B. Brooks, C. C. Alley, and J. A. Liddle, Anal. Chem. 46:1930–1934, 1974; J. B. Brooks, D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley, J. Clin. Microbiol. 11:45–51, 1980; J. B. Brooks, D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley, J. Clin. Microbiol. 11:52–58, 1980). The results were different from previous FPEC GLC profiles of SR and CSF from patients with known tuberculous meningitis. Both the SR and CSF contained several unidentified compounds that were not previously detected in tuberculous meningitis or any of our other studies of body fluids. *Nocardia brasiliensis* was later isolated from the patient. Detection of these metabolites by FPEC GLC could prove to be useful for rapid diagnosis of *Nocardia* disease, and their identification will provide a better understanding of metabolites produced by *Nocardia* sp. in vivo.

Nocardia sp. is an uncommon cause of meningitis, and *Nocardia* meningitis has a clinical picture similar to that of tuberculous meningitis (7). Early detection and specific therapy for meningitis caused by *Nocardia* sp. or *Mycobacterium tuberculosis* can significantly affect the prognosis of a patient.

Frequency-pulsed electron capture gas-liquid chromatography (FPEC GLC) analysis of cerebrospinal fluid (CSF) has been used to aid in the rapid identification of tuberculous meningitis (4). Our recent detection of tuberculostearic acid (TSA) in both CSF and serum (SR) of patients with tuberculous meningitis holds promise for an improved FPEC GLC test with increased specificity for detection of tuberculous meningitis (unpublished data).

Samples of CSF and SR from a patient with suspected tuberculous meningitis were sent to our laboratory from the HCA Aiken Regional Medical Centers in Aiken, S.C. (HARMC), to be analyzed by FPEC GLC for tuberculous meningitis. After our analysis, we reported negative results for tuberculous meningitis but noted the presence of metabolites not previously found in tuberculous meningitis patients or controls. *N. brasiliensis* was subsequently cultured at HARMC from the patient, and the whole spent culture medium from this isolate was sent to our laboratory for analysis and in vivo-in vitro comparison of metabolites. If the FPEC GLC analysis of SR, CSF, and spent culture media showed a correlation in metabolites, the peaks detected in the SR and CSF may provide markers for rapid identification of *Nocardia* meningitis by FPEC GLC, the reason being that in vitro-in vivo correlation of metabolites presents strong evidence that the metabolites originated from the infectious agent.

CSF and SR (2 ml each) that had not been centrifuged were extracted with high-purity nanograde solvents under acid and basic conditions as previously described (4) to obtain carboxylic acids, hydroxy acids, and amines. Carboxylic acids were esterified with trichloroethanol (TCE), amines were derivatized with heptafluorobutyic anhydride, and hydroxy acids were diesterified with heptafluorobutyric anhydride-ethanol as described previously (1, 3). Derivatized amines and hydroxy acids were analyzed with a Perkin-Elmer 3920 FPEC GLC on a glass column (2 mm [inside diameter] by 7.6 m) that had been packed with OV-101 on 80/100 mesh Chromosorb W (5, 6). Trichloroethanol derivatives of carboxylic acids were analyzed by FPEC GLC on the same type of instrument equipped with a large-bore, nonpolar, fused-silica capillary column (0.5 mm [inside diameter] by 10 m) coated with a 4.4-μm-thick film of OV-1. Carboxylic acid derivatives were also analyzed on a polar fused-silicia capillary column (0.32 mm [inside diameter] by 25 m) coated with a 0.25-μm-thick film of OV-225. Helium was used as a carrier gas in both capillary columns at a flow rate of 5 ml/min. The makeup gas for the capillary columns was 95% argon–5% methane. The combined flow rate of the carrier and makeup gases through the detector was 70 ml/min. For analysis of trichloroethanol-derivatized acids on the nonpolar column, the instrument was programmed from 175 to 275°C at a linear increase of 2°C/min. When using the polar column, we held the instrument at 100°C for 3 min and then programmed it to 220°C at a linear increase of 2°C/min. A 1-μl sample was used for each analysis. An IBM 9000 computer equipped with Cap 1.3

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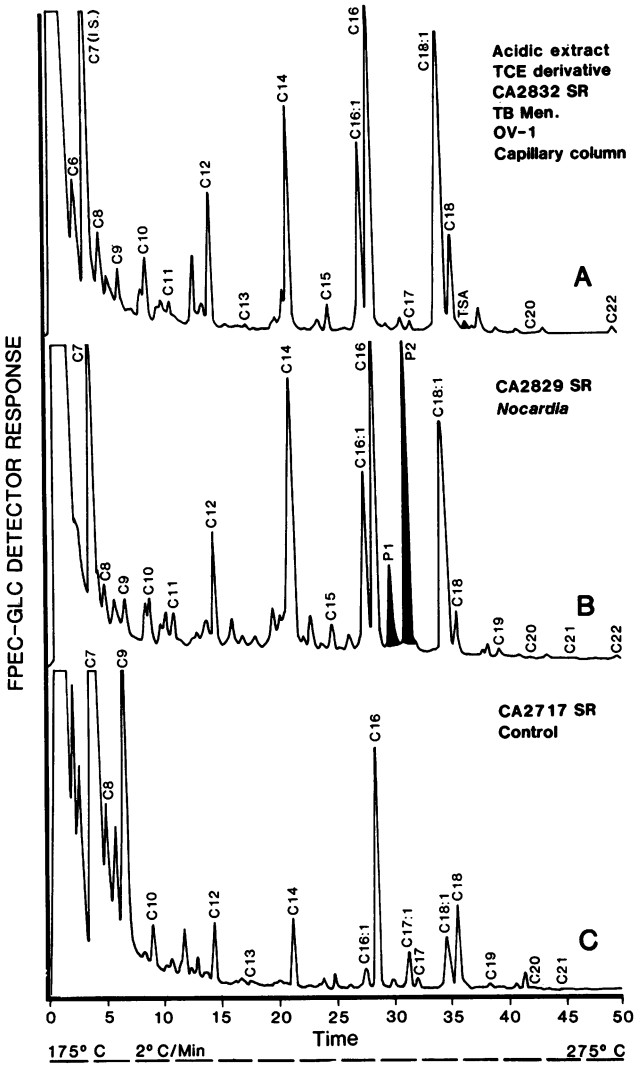

FIG. 1. FPEC GLC chromatograms of trichloroethanol derivatives prepared from acidic chloroform extracts of SR. Analyses were performed on an OV-1 large-bore capillary column. The letter C followed by a number indicates a saturated carboxylic acid with the number of carbon atoms indicated by the number. A colon between two numbers indicates unsaturation. P followed by a number indicates an unidentified compound. Abbreviations: I.S., Internal standard; TB men., tuberculous meningitis. CA numbers are computer entry numbers, and SR and CSF from the same patient were given consecutive CA numbers.

software was used to integrate the peaks, expand sections of the chromatogram for easy comparison, and adjust peak attenuation.

Distinctive differences were observed in the carboxylic acids of SR from the patient we were testing with a culture-proven *Nocardia* infection and SR from patients with culture-proven tuberculous meningitis (Fig. 1A, B, and C, blackened peaks). TSA, which eluted just after C18 at 35:3 min (Fig. 1A, blackened peak), was found in SR from patients with tuberculous meningitis. TSA has been reported to be a component of the cell wall of *N. brasiliensis* (2), but it was not detected in either the in vivo or in vitro studies performed by us. Unidentified peaks labeled P1 and P2 (Fig. 1B) were present in the SR of the patient with nocardiosis, but these were absent in controls and in SR from patients with tuberculous meningitis. Peak 2 was also detected in spent culture medium of the *Nocardia* isolate cultured from the patient (Fig. 2B). Peak 2 from the patient SR and peak 2 from the spent culture media gave a perfect retention time match on both a high-resolution nonpolar column and a high-resolution polar capillary column (Fig. 2A and B, blackened peaks). Detection by FPEC GLC of peak 2 in the spent culture medium, in which disease-causing *Nocardia* sp. was grown, along with its detection by FPEC GLC in the SR of the patient from which the isolate was obtained, gave strong indications that peak 2 was associated with the *Nocardia* infection. Since peak 2 was not found in the SR of controls or patients with tuberculous meningitis, its detection in SR, along with the absence of TSA, could be a significant marker for differentiating tuberculous meningitis from meningitis caused by *N. brasiliensis* and perhaps other *Nocardia* species. Further application of the technique to

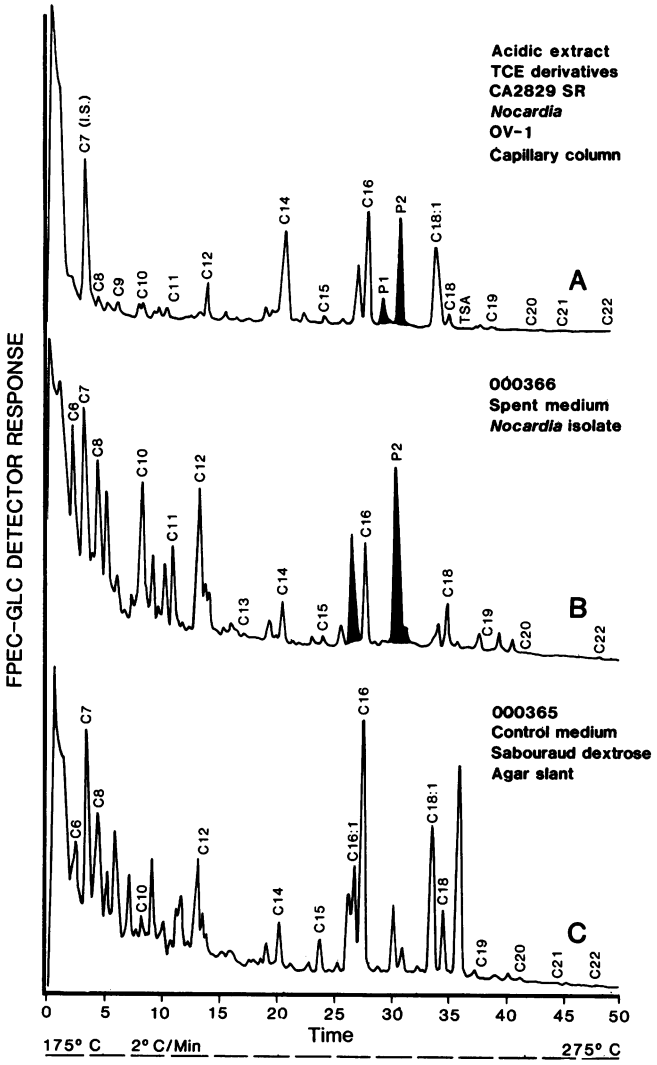

FIG. 2. FPEC GLC chromatograms of TCE derivatives prepared from acidic chloroform extracts of SR and spent and control culture media. The FPEC GLC conditions were as described in the legend to Fig. 1. For definitions of the abbreviations, see the legend to Fig. 1.

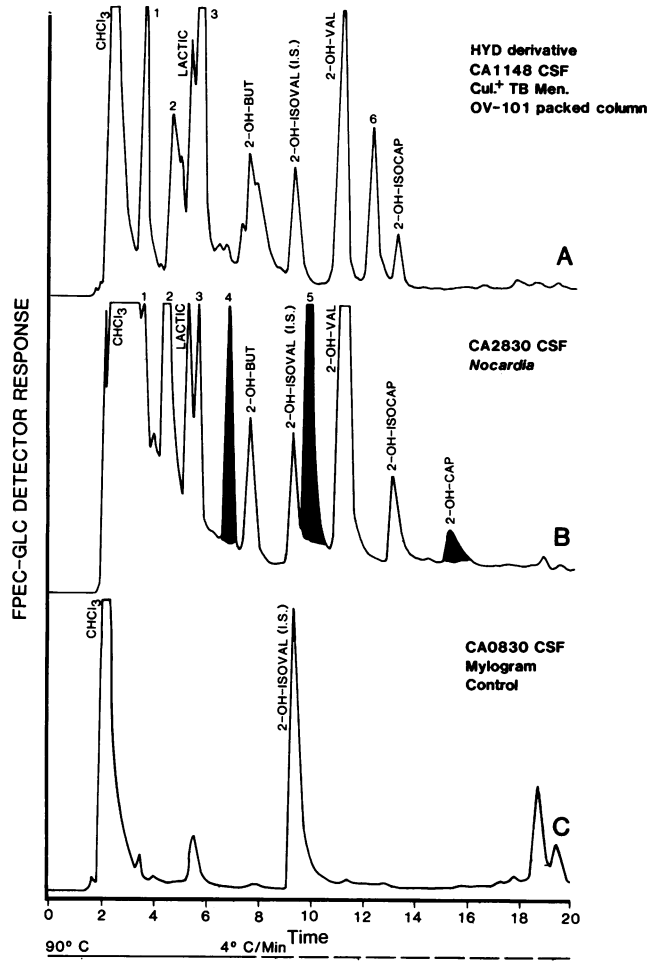

FIG. 3. FPEC GLC chromatograms of heptafluorobutyric anhydride-ethanol derivatives prepared from acidic ethyl ether extracts of CSF. Analyses were performed on an OV-101-packed column. Abbreviations: BUT, Butyric; VAL, valeric; ISOVAL, isovaleric; ISOCAP, isocaproic; CAP, caproic; Cul.+, culture proven. For the other abbreviations, see the legend to Fig. 1.

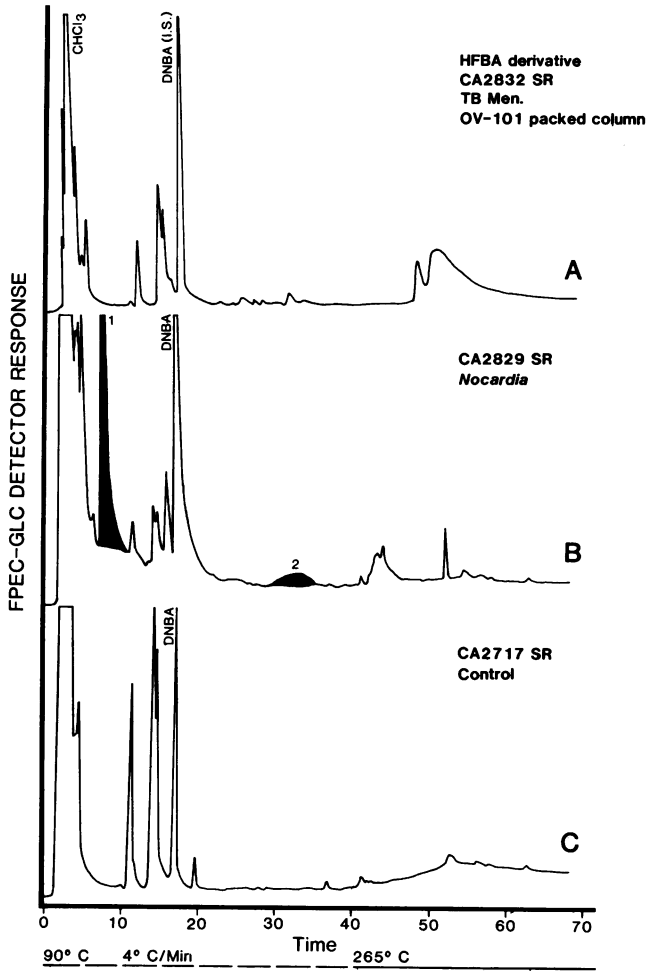

FIG. 4. FPEC GLC chromatograms of heptafluorobutyric anhydride derivatives prepared from basic chloroform extracts of SR. Abbreviations: I.S., Internal standard; DNBA, di-*n*-butylamine. Analyses were performed on an OV-101-packed column. For other abbreviations, see the legend to Fig. 1.

analysis of additional clinical specimens will be necessary for determination of its true value.

The carboxylic acid FPEC GLC profile obtained by analysis of CSF from the patient with nocardiosis did not contain significant peaks. However, the absence of TSA in the CSF of the nocardiosis patient is significant for differentiation of nocardiosis from tuberculous meningitis. The most significant peaks detected in the CSF of the nocardiosis patient were found in the hydroxy acid analysis (Fig. 3B, blackened peaks [no. 4 and 5 and 2-hydroxycaproic acid]). The amines (Fig. 4B, blackened peaks) detected in the SR of the patient with nocardiosis were also different from those obtained by FPEC GLC analysis of SR and CSF from controls and patients with tuberculous meningitis (Fig. 3A, B, and C and 4A, B, and C); both the hydroxy acids and amines may prove to be important markers for identification of *Nocardia* disease. However, hydroxy acids and amines were not detected in vitro, and their possible association with *Nocardia* infection must await further FPEC GLC studies involving SR and CSF from several patients with *Nocardia* infection or further in vitro studies involving different culture conditions.

LITERATURE CITED

1. **Alley, C. C., J. B. Brooks, and D. S. Kellogg, Jr.** 1977. Electron capture gas-liquid chromatographic-mass spectral identification of acids produced by *Neisseria meningitidis* in a defined medium. J. Clin. Microbiol. **9**:97–102.
2. **Bordet, C., and G. Michel.** 1963. Studies on the fatty acids of several *Nocardia* species. Biochim. Biophys. Acta **70**:613–626.
3. **Brooks, J. B., C. C. Alley, and J. A. Liddle.** 1974. Simultaneous esterification of carboxyl and hydroxyl groups with alcohol and heptafluorobutyric anhydride for analysis by gas chromatography. Anal. Chem. **46**:1930–1934.
4. **Brooks, J. B., D. C. Edman, C. C. Alley, R. B. Craven, and N. I. Girgis.** 1980. Frequency-pulsed electron capture gas-liquid chromatography and the tryptophan color test for rapid diagnosis of tuberculous and other forms of lympocytic meningitis. J. Clin. Microbiol. **12**:208–215.
5. **Brooks, J. B., D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley.** 1980. Rapid differentiation of the major causative agents of bacterial meningitis by use of frequency-pulsed electron capture gas-liquid chromatography: analysis of acids. J. Clin. Microbiol. **11**:45–51.
6. **Brooks, J. B., D. S. Kellogg, Jr., M. E. Shepherd, and C. C. Alley.** 1980. Rapid differentiation of the major causative agents of bacterial meningitis by use of frequency-pulsed electron capture gas-liquid chromatography: analysis of amines. J. Clin. Microbiol. **11**:52–58.
7. **Ellner, J. J., and J. E. Bennett.** 1976. Chronic meningitis, p. 350–351. The Williams & Wilkins Co., Baltimore.